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Publication date:
2018

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Nielsen, M. R., Pedersen, T. B., Wollenberg, R., Søndergaard, T. E., & Sørensen, J. L. (2018). *Identification of the first steps in phenalenone pigment biosynthesis in Fusarium solani*. 100. Poster presented at European Fusarium Seminar 14, Austria.

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Identification of the first steps in phenalenone pigment biosynthesis in *Fusarium solani*

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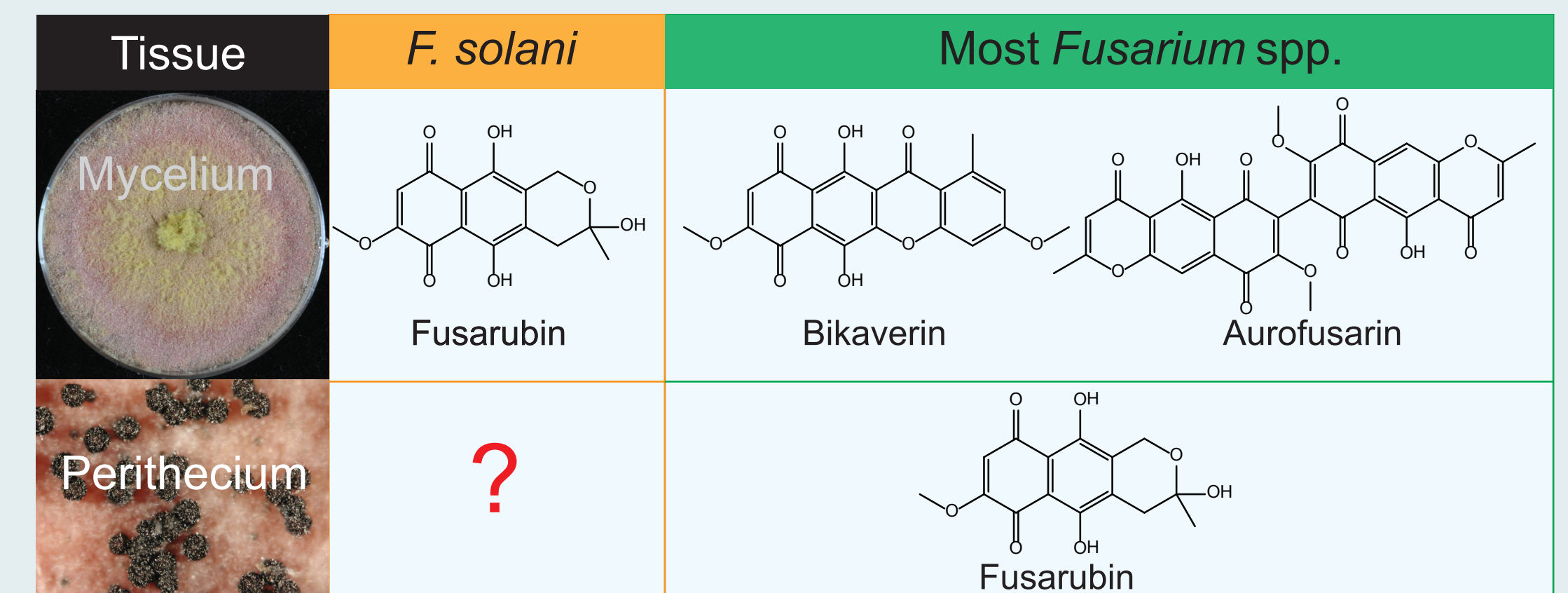
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BACKGROUND - *FUSARIUM* PIGMENTS

- Most *Fusarium* Species produce bikaverin and aurofusarin for mycelium pigmentation and fusarubins for perithecial pigmentation [1].
- Fusarium solani* produces fusarubins during mycelial growth and another unknown pigment during sexual reproduction. This unknown pigment is predicted to be synthesized by a non-reducing polyketide synthase (PKS35 = *pksN* [2]).
- The aim of this study is to identify the pigment through heterologous production in yeast.



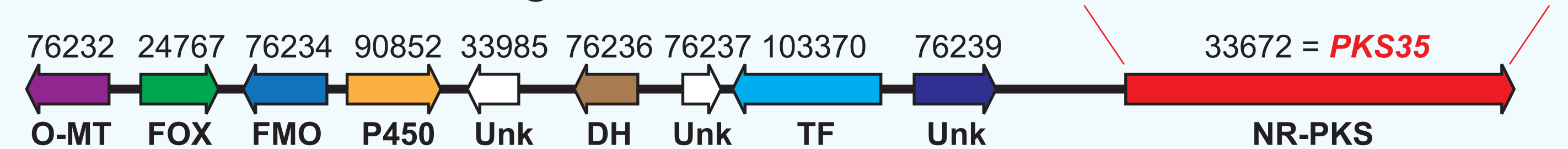
Pigments in *Fusarium*

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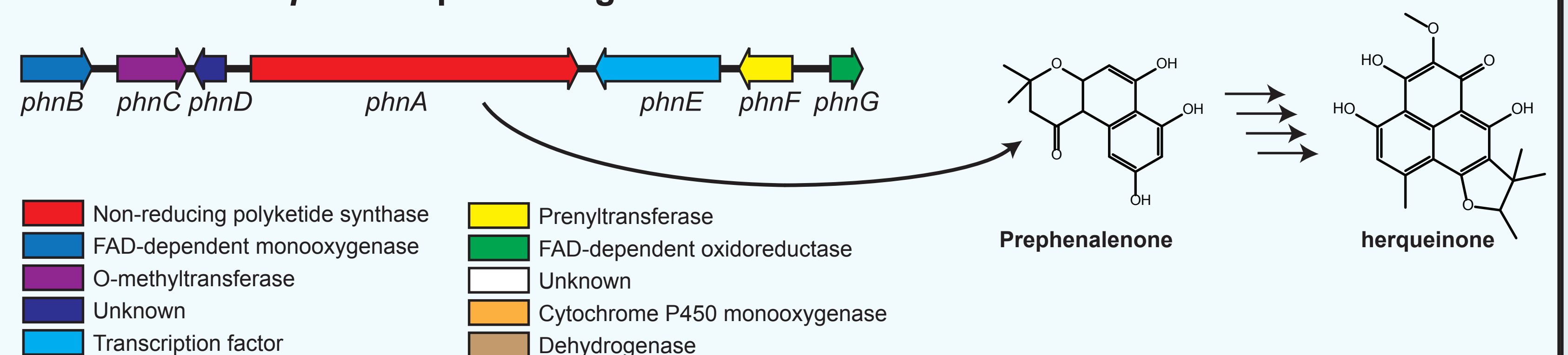
PKS35 GENE CLUSTER

- Comprised of 10 genes (NECHADRAFT_76332 - 33672)
- Orthologs of six genes are also present in the herqueinone cluster
- The first step in herqueinone biosynthesis is prephenalenone, which is cyclized to a tricyclic phenalenone ring structure by a FAD-dependant monooxygenase [3].

Fusarium solani PKS35 gene cluster



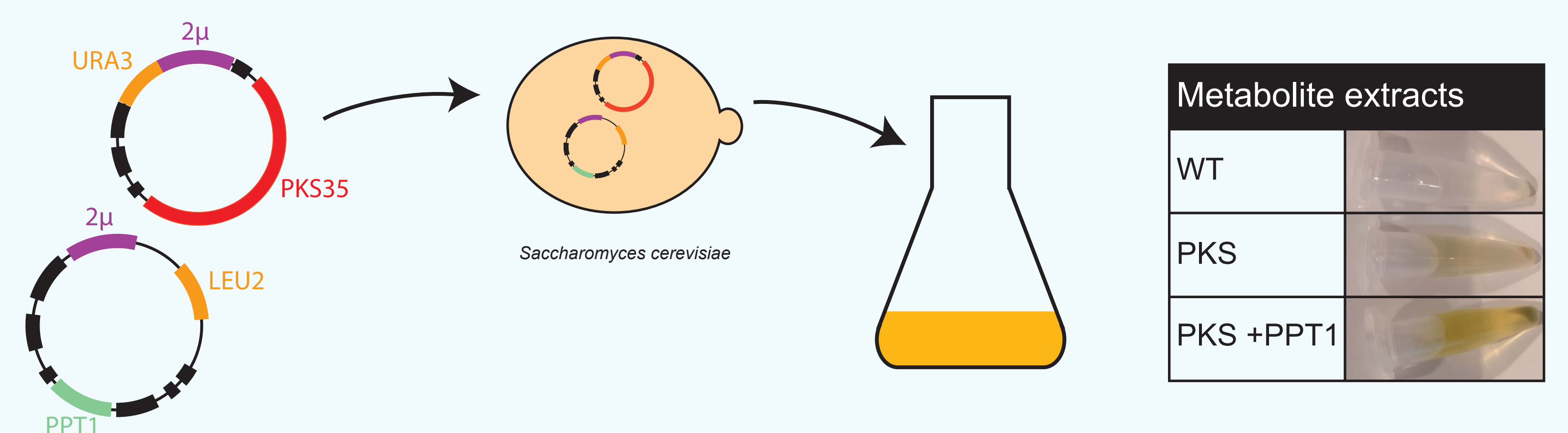
Penicillium herquei herqueinone gene cluster



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HETEROLOGEOUS PRODUCTION

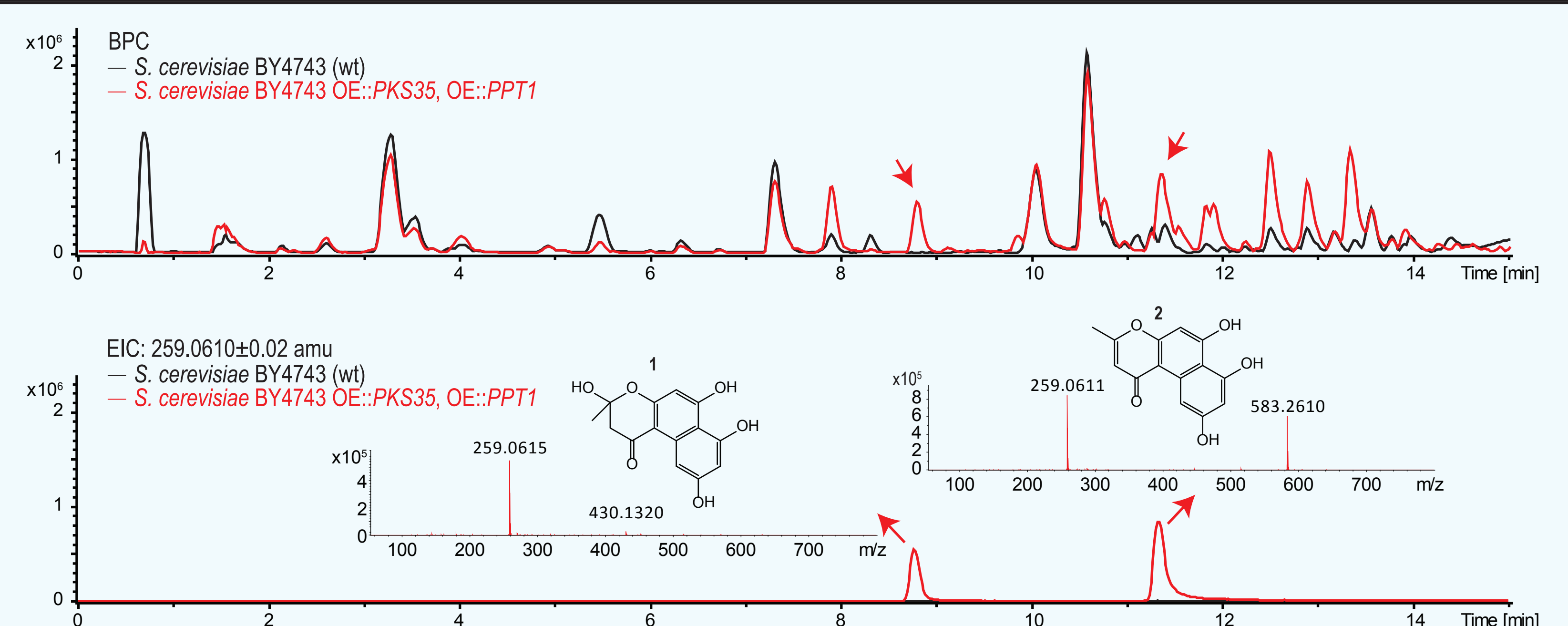
- Intronless *PKS35* was cloned into a 2μ vector and put under control of a galactose inducible promoter *P_{GAL1}*.
- A Sfp-Type 4'-Phosphopantetheinyl Transferase (*PPT1*) was also expressed from another 2μ vector to facilitate polyketide formation.
- The transformed yeast strain was cultivated under induced conditions in liquid cultures for five days.



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IDENTIFICATION OF PKS PRODUCT

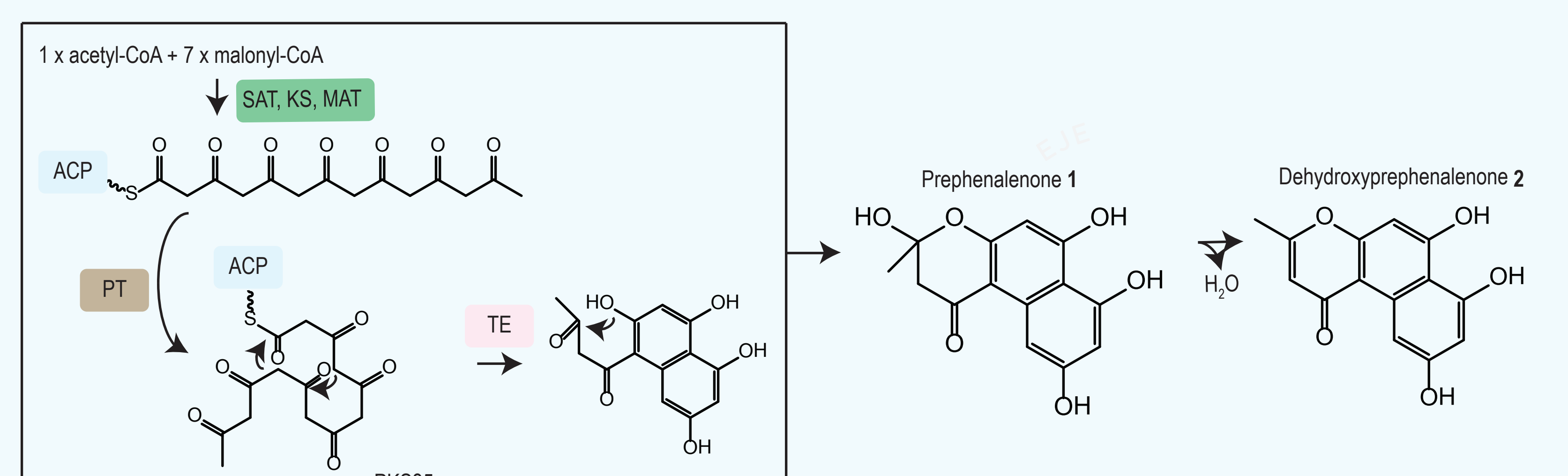
- Production of secondary metabolites was analyzed by high-resolution mass spectrometry (HRMS).
- The yeast strain produced prephenalenone; the first step of the herqueinone pathway [3].
- We also detected dehydroxyphenalenone, which is formed by spontaneous dehydration.



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BIOSYNTHETIC PATHWAY AND OUTLOOK

- PKS35 produce prephenalenone from a single acetyl-CoA and seven malonyl-CoA units
- Prephenalenone is expected to be cyclized and additionally modified to a phenalenone pigment in *F. solani*.
- Future experiments will include heterologous production of additional genes in yeast and constitutive expression of the TF in *F. solani*



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